



DELAWARE LABORATOR

SPRING

2008

PULSED-FIELD GEL ELECTROPHORESIS (PFGE) TESTING AT DELAWARE PUBLIC HEALTH LABORATORY

Jennifer Cascarino, Microbiologist II and Jack S-C. Liou, Ph.D Laboratory Manager II

The importance of Pulse-field gel electrophoresis (PFGE) at DPHL was recently demonstrated in three significant outbreaks. In the fall of 2006, approximately 71 people were involved in a multi-state outbreak of *E. coli* O157:H7 (4). Five states (Delaware, New Jersey, New York, Pennsylvania, and South Carolina) reported this illness to the CDC and the outbreak was clearly linked to Taco Bell restaurants in the northeastern United States. 75 percent of the ill patients were hospitalized and 11percent developed hemolytic-uremic syndrome (HUS), confirming the seriousness of *E. coli* O157:H7 infections. The majority of the cases had the same PFGE DNA fingerprint, confirming the outbreak (4).

Pulsed-field gel electrophoresis (PFGE) allows the separation of DNA varying in size from a few kilobase pairs to 10 megabase pairs (2). PFGE was first described in 1984 by Schwartz and Cantor, in increasing the size limit of DNA separation in an agarose gel (7). PFGE has been successfully used to determine the molecular epidemiology of numerous organisms and is considered the gold standard for bacterial subtyping (1). DNA from submitted bacterial specimens is prepared in agarose plugs. Whole cells from the organism are embedded in the agarose plug then lysed, allowing purification of chromosome-sized DNA without shearing (2). The proper cell concentration is needed when preparing cells for embedding in the agarose. Cell concentration is determined using a colorimeter. After the cells are lysed, the agarose plugs are cut using site-specific restriction enzymes (XbaI) (8). Restriction enzymes cut

double-stranded DNA at specific sequences (Fig. 1).

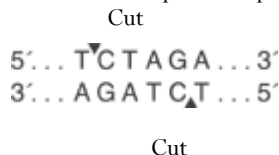


Fig. 1. XbaI Restriction Endonuclease Recognition Site

A second restriction with the enzyme AvrII can also be used with certain organisms. Restricted DNA fragments are then separated by electrophoresis using a CHEF (contour-clamped homogeneous electric field) Mapper (7). PFGE differs from standard gel electrophoresis because the DNA is separated by periodically changing the direction of the electric field electronically to reorient the DNA by changing the polarity of an electrode array (7). Larger DNA lags behind, providing a separation from smaller DNA which moves faster (7). The DNA fragments are stained with ethidium bromide and an image of the gel is captured with a camera. The image shows the bands/fingerprints unique to each organism and serotype (Fig. 2).

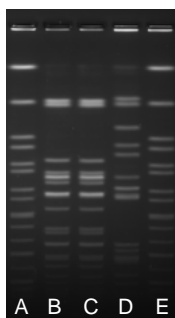


Fig. 2. Example of a *Salmonella* PFGE gel image. Lanes A and E: Standard isolate (*Salmonella* Braenderup H9812); Lane B: Patient #1 isolate (*Salmonella* paratyphi a); Lane C: Patient #2 isolate (*Salmonella* paratyphi a); Lane D: Patient #3 isolate (*Salmonella* typhimurium). Lanes B and C have the same exact band pattern.



Inside this issue:

Pulsed-Field Gel Electrophoresis (PFGE) Testing at Delaware Public Health	1
What is CLIA ... and Why is it Important for Laboratories?	3
Training at DPHL	4
Employee News	4

Special Points of Interest

Front Page Article: Pulsed-Field Gel Electrophoresis (PFGE) Testing at Delaware Public Health Laboratory

What is CLIA ... and Why is it Important for Laboratories?

Page 3

Training at DPHL

Page 4

PFGE Testing at DPHL, continued from page 1

SPECIMEN SUBMISSION AND RESULT REPORTING

Specimens are submitted to the clinical microbiology lab for confirmation and further grouping/serotyping of isolates. Grouping is performed on all *Shigella*, *Salmonella*, and shigatoxin-producing *E. coli* isolates. *Salmonella* and *E. coli* are serotyped using somatic and flagellar antisera. After serotyping is completed, the microbiology lab is able to further characterize these isolates using PFGE. After PFGE is done, the DNA fingerprints generated (Fig 2.) from each isolate are sent to the CDC where they are placed in the national PulseNet database. PulseNet is the national molecular subtyping network for foodborne disease surveillance (5). The PulseNet database is electronically available to participants, allowing for rapid comparison of fingerprint patterns (5). Indistinguishable and closely related (differing by no more than one band) isolates are compared in the PulseNet database and linked in outbreaks. Epidemiological information is also sent to the CDC along with the isolates to help link outbreaks. Comparing the DNA fingerprint and the epidemiological information makes it easier to identify disease clusters involving several states. PulseNet facilitates real-time communication among state, local health departments, and international partners. It also helps food regulatory agencies recognize areas where improvement is needed to increase the safety of our food supply (5).

SIGNIFICANCE OF PFGE

Delaware was the first state to send PFGE results from the Taco Bell outbreak to the CDC (9). *E. coli* O157:H7 is a mandatory reportable condition in Delaware and the Epidemiology section is responsible for investigating *E. coli* cases in the state. After reports of a possible Taco Bell outbreak in the northeast United States, the Bureau of Epidemiology realized that Delaware had a possible *E. coli* case linked to the outbreak (9). This allowed for DPHL to promptly perform PFGE on the suspected isolate and send out the *E. coli* outbreak fingerprint. After confirmation of cases in Delaware, Taco Bell restaurants were inspected, sanitized, and food supplies were discarded. Also, restaurants with suspected associated cases had all employees tested for *E. coli* presence in their stool. DPHL also tested

food samples from one Taco Bell restaurant where there was a suspected case. Real-time PCR (polymerase chain reaction) was performed on 19 samples including lettuce, tomato, white onions, cilantro, cheese, and salsa. The stool and food samples were negative for *E. coli* (9). In 2007, ConAgra recalled its Banquet and generic store brand frozen (not-ready-to-eat) pot pie products due to reported *Salmonella* illnesses linked to their product (6). Delaware had confirmed cases involved in this outbreak. The CDC contacted DPHL to run further PFGE testing on suspected isolates. The outbreak serotype was identified as *Salmonella* 1, 4, 5, 12: i -. More recently, April 5, 2008, the Malt-O-Meal Company recalled its unsweetened Puffed Rice and Wheat Cereals after routine food testing detected *Salmonella* in their products (3). Two days later, the CDC PulseNet team identified a cluster of human *Salmonella agona* isolates with indistinguishable PFGE patterns in multiple states. The human PFGE patterns matched the PFGE pattern found at the Malt-O-Meal plant (3). Delaware had two confirmed cases from this outbreak and was able to obtain the recalled cereal from one patient. *S. agona* was successfully isolated from the puffed rice product. The PFGE pattern isolated from the cereal was indistinguishable from the patient PFGE pattern.

These three outbreaks demonstrate the importance of PFGE testing at DPHL to investigate foodborne outbreaks. It was evident that cooperation and quick testing by DPHL (using PFGE) and the Bureau of Epidemiology helped ensure that the outbreak was controlled and further cases were prevented in the state of Delaware.

References

1. Barrett, T.J., Lior, H., Green, J.H., Khakhria, R., Wells, J.G., Bell, B.P., Greene, K.D., Lewis, J., and Griffin, P.M. 1994. *Laboratory Investigation of a Multistate Foodborne Outbreak of Escherichia coli O157:H7 by Using Pulsed-Field Gel Electrophoresis and Phage Typing*. Journal of Clinical Microbiology. 32(12): 3013-3017.
2. Bio-Rad Laboratories. *CHEF Genomic DNA Plug Kits Instruction Manual*. LIT510 Rev. E.
3. CDC. 2008. *Investigation of Outbreak of Infections Caused by Salmonella Agona*. Salmonella CDC. <<http://www.cdc.gov/salmonella/agona/>>

salmonella/agona/>

4. CDC. 2006. *Multistate Outbreak of E. coli O157 Infections, November-December 2006*. Foodborne and Diarrheal Diseases Branch. <<http://www.cdc.gov/ecoli/2006/december/121406.htm>>
5. CDC. 2006. *PulseNet*. <<http://www.cdc.gov/pulsenet/>>
6. Eamich, A. 2007. *FSIS Issues Public Health Alert for Frozen Chicken and Turkey Pot Pies*. USDA FSIS News Releases. <http://www.fsis.usda.gov/News_&_Events/NR_100907_01/index.asp>
7. Joppa, B., Li, S., Cole, S., and Gallagher, S. 1993. *Pulsed Field Electrophoresis for Separation of Large DNA*. HSI Laboratories. <<http://www.nal.usda.gov/pgdic/Probe/v2n3/puls.html>>
8. Mahon, B.E., Ponka, A., Hall, W.N., Komatsu, K., Dietrich, S.E., Stonen, A., Cage, G., Hayes, P.S., Lambert, M.A., Bean, N.H., and Griffin, P.M. 1997. *An International Outbreak of Salmonella Infections Caused by Alfalfa Sprouts Grown from Contaminated Seeds*. The Journal of Infectious Diseases. 175: 876-882.
9. Shore, S. *Anatomy of a Foodborne Outbreak*. Delaware Laborator. 31(3):1-8.



New!!!

Please take a moment and respond to our laboratory services survey

LPAC page

**More Laboratory Section Reports
Revised Test Requisition Form
Specimen Collection Procedures**

www.dhss.delaware.gov/dhss/dph/lab/labs.html

WHAT IS CLIA... AND WHY IS IT IMPORTANT FOR LABORATORIES?

Fred Franze, Quality Assurance Lab Manager

The purpose of CLIA is to set minimum standards for all laboratories to follow and to determine if laboratories are achieving those standards. The acronym "CLIA" stands for the Clinical Laboratory Improvement Amendments. Congress passed these amendments in 1988 establishing quality standards for all clinical laboratory testing to ensure the accuracy, reliability and timeliness of patient test results.

CLIA began in the late 1960's when problems arose in the cytology laboratories that read PAP smears. The personnel in these laboratories were overworked and had a very high error rate. Many women suffered or died because the cytologists had missed the early stages of cancer on the PAP smears. In 1967, the Clinical Laboratory Improvement Act was passed and the first laboratory regulations were born. The amendments to this Act, though passed in 1988, did not go into effect until February 28, 1992 when the new regulations were approved and published in the Federal Register.

The CLIA requirements are based on the complexity of the tests performed and not on the type of laboratory where the testing is performed. CLIA Brochure #5, "How to Obtain a CLIA Certificate", states that CLIA

requires all facilities that perform even one test, including waived tests (see Certificate of Waiver, below), on "materials derived from the human body for the purpose of providing information for the diagnosis, prevention, or treatment of any disease or impairment of, or the assessment of the health of, human beings" to meet certain federal requirements. If a facility performs tests for these purposes, it is considered a laboratory under CLIA and must apply and obtain a certificate from the CLIA program that corresponds to the complexity of tests performed. The Centers for Medicare and Medicaid Services (CMS) is responsible for ensuring CLIA compliance and administering the program. There are different types of certificates, all of which are effective for two years:

◆ **Certificate of Waiver (COW)** is issued to a laboratory that performs only waived tests. A waived test is categorized as simple laboratory examinations and procedures that have an insignificant risk of an erroneous result. The FDA determines if the test meets the criteria for waived status. An example of a waived test would be a urine pregnancy test.

◆ **Certificate for Provider Performed Microscopy (PPM) Procedures** is issued to a laboratory in which a physician or midlevel practitioner performs specific microscopy procedures during the course of a patient's visit. The primary instrument used to conduct these tests is the microscope, and the procedures authorized are categorized as moderately complex.

◆ **Certificate of Compliance** is issued to a laboratory that is conducting moderate and highly complex testing. This certificate is only issued after the laboratory has passed an on-site inspection that is conducted to ensure that the lab is compliant with all applicable CLIA requirements. Moderate and highly complex tests require a high degree of skill, training and education for accuracy. This type of testing is now referred to as "Nonwaived".

The Delaware Division of Public Health Laboratory (DPHL) holds two Certificates of Compliance. One certificate covers the main

laboratory to perform testing in the following specialties: virology, molecular microbiology, bacteriology, parasitology, TB testing, newborn screening and blood lead analysis. The second certificate covers the 11 satellite labs located in the various Division of Public Health clinic facilities located throughout the state. Testing at these sites includes tests for sexually transmitted diseases (std) and std and pregnancy testing for family planning. The Director of the Public Health Laboratory is listed as the responsible individual for both of these certificates.

The following DPHL employees play an integral role with regards to CLIA compliance of laboratories located in Delaware:

► **The quality assurance manager** (QA) is responsible for monitoring the quality assessment functions utilized by the various DPHL specialties to ensure compliance with CLIA requirements. Some of these functions include proficiency testing, identification and resolution of testing errors, personnel qualifications and training as well as quality control procedures. Additionally, the QA manager is responsible for providing technical oversight to the 11 satellite sites throughout the state.

► **The CLIA compliance officer** is responsible for inspecting all laboratories in the state that hold a Certificate of Compliance, and a certain percentage of labs that hold a Certificate of Waiver or Certificate for PPM Procedures. Additionally, this office will investigate any complaints received about any CLIA certified lab throughout the state. The compliance officer is required to pass an annual audit conducted by CMS to ensure that inspections, documentations and follow-ups are done correctly and in a timely fashion.

Recently, the Delaware Public Health Laboratory was inspected by the Certification and Enforcement branch of CMS (region 3). We are once again proud to announce that we passed our inspection and have been re-certified. For further information about CLIA see the DPHL web site <http://www.dhss.delaware.gov/dhss/dph/lab/clia.html>



Governor Ruth Ann Minner signs the proclamation designating April 20-25 Laboratory Professionals Week. Dr. Jane Getchell, DPHL Director (1st from right) and Dr. Jack Liou, Laboratory Manager II (2nd from right back row), attended the ceremony. The Lab celebrated with special events including tours by two area schools.

TRAINING AT DELAWARE PUBLIC HEALTH LABORATORY

Kathy Gray, Chemist III, Training Coordinator

Due to the unpredictable nature of working in a scientific environment, technology and information is ever changing. Continuing education is a must so that we can keep up with the latest in test methodology, equipment, policies, procedures, and threats to the health of our citizens. As a member of the Association of Public Health Laboratories (APHL) the Delaware Public Health Laboratory is eligible to participate in a multitude of training opportunities throughout the year.

Working in conjunction with APHL, other organizations such as the National Laboratory Training Network (NLTN) and Clinical and Laboratory Standards Institute (CLSI) offer courses at various labs throughout the country. The NLTN holds regional training sessions organized by both the NLTN and the host state lab.

Delaware, in partnership with APHL, recently hosted a 'wet' workshop for sentinel labs on May 1 and 2, 2008 focusing on bioterrorism agents. A 'wet' workshop is one in which attendees gain hands-on experience through exercises based on case studies using "mimic" and real agents.

Another type of course offering is through teleconferences—a highly effective mode of reaching a geographically diverse audience, with a question/answer segment at the end. A calendar of teleconference courses offered by NLTN and APHL is usually published six months in advance and is available on the APHL website. These organizations also offer many courses via webcast and archive many presentations on CD, which are available to participants upon request or registration.

It is the goal of the Delaware Public Health Lab to make as many of these opportunities available not only to the laboratorians at DPHL, but also our sentinel lab partners and other professionals throughout the state who are charged with protecting the health and safety of Delawareans. Notification of course opportunities will be available by email. Those wishing to be included in the email should call the lab at 302-223-1520 or email Kathy.Gray@state.de.us. A list of APHL and NLTN courses is available at the link displayed below:

<https://www.aphl.net.org/eweb/DynamicPage.aspx?Site=aphl&webcode=TrainEventList>

Information on teleconferences can be found at: <http://www.dhss.delaware.gov/dhss/dph/lab/learninglinks.html>.

EMPLOYEE NEWS

The Laboratory extends a heartfelt "Happy Retirement" to **Susan Everett** who retired effective April 30, 2008, after 39 years of service. Susan was employed as a laboratory technician III in the environmental chemistry section performing water testing. We'll miss you Susan and we wish you a joyful retirement!



Welcome to **Carrie Paquette-Straub**, microbiologist III, who joined the lab on March 3, 2008. Carrie has a Masters in Science from the University of Vermont, with a focus on Cell and Molecular Biology. She spent the past 9 years at the University of Delaware, working in melanoma research. Carrie and her husband, Dylan, have a beautiful 3 ½ year old daughter, Rachel, as well as two dogs, a cat and 9 fish. Her interests include gardening, reading and doing Body Shop at Home parties.

Leslie Jones hails from Norfolk State University in Norfolk, VA where she obtained a B.S. in Biology. Leslie began her employment at the Lab on March 3, 2008 as a laboratory technician III. She is currently working at Porter State Service Center performing phlebotomy and STD testing. Leslie was previously employed with Quest Pharmaceutical Services as a staff scientist. Welcome, Leslie!



Delaware's Division of Public Health Laboratory
30 Sunnyside Road
Smyrna, DE 19977
302.223.1520
Fax: 302.653.2877



Jaime "Gus" Rivera, MD, FAACP
Director, Delaware's Division of Public Health

Christina Pleasanton, MS
Deputy Director, Delaware Public Health Laboratory

*"To Protect and Enhance the Health
of the People of Delaware"*

Jane P. Getchell, DrPH Director,
Delaware Public Health Laboratory

If you have questions regarding these articles or would like to receive a hard copy of this newsletter, contact the Delaware Public Health Laboratory at 302.223.1520. To receive this newsletter by email, contact liz.moore@state.de.us.

Document Control #
35-05-20/08/04/75